Exploitation of new attractants for fruit fly pests of economic importance

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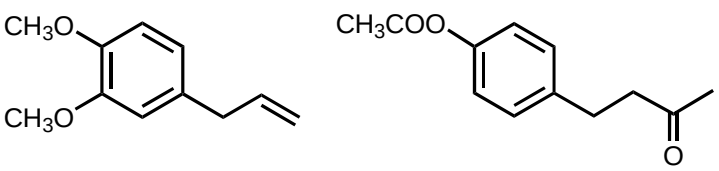
ABSTRACT

The fruit fly tribe Dacini (Tephritidae: Diptera) comprises more than 700 species including many fruit pests of economic importance, particularly in the two genera, *Bactrocera* and *Zeugodacus*. Males of many dacine species show strong affinity either to methyl eugenol (ME) or cue-lure (CL). However, there are over a hundred species that show no affinity to these lures. We have been seeking new attractants for these 'nonresponding' species particularly of endemic agricultural importance based on the following three criteria, (1) by observing their mating sequences, (2) their natural interactions with plants and (3) by analyzing specific chemicals the wild male flies accumulate in their secretory organ (rectal gland) associated with pheromone production. Orchid flowers of many *Bulbophyllum* species attract fruit fly males by emitting specific volatiles for pollination, in the rain forests of Southeast Asia. Several species produce either ME or raspberry ketone (RK), and some species use zingerone (ZN) to attract both ME-sensitive and RK-sensitive males. ZN was found as a specific male attractant for *B. jarvisi*. A specific attractant for the solanaceous fruit fly, *B. latifrons*, was characterized as 3-hydroxy-α-ionone from a specific variety of eggplants. Several 3-oxygenated α-ionone analogs were developed as monitoring agents for *B. latifrons* in Okinawa, Japan. Furthermore, wild *Bactrocera* males that have fed on attractants from certain plant sources selectively accumulate/biotransform compounds in the rectal gland possibly for sex pheromone to attract females. A sesquiterpene, β-caryophyllene, was identified from the rectal gland in wild *B. correcta* males. β-Caryophyllene was found to be more attractive than ME to males, and thus, potentially serves as a new monitoring agent for *B. correcta*. These exemplify the importance of a multidisciplinary study including behavioral, physiological, biochemical and chemoecological aspects to develop new fruit fly attractants.

Keywords: *Bactrocera*, *Zeugodacus*, Dacini, Tephritidae, Diptera, fruit fly, methyl eugenol, cue-lure, raspberry ketone, zingerone, 3-oxo-α-ionone, β-caryophyllene, phenylpropanoid, phenylbutanoid, terpenoid, attractant, pheromone, allomone, synomone, floral fragrance, rectal gland, fruit fly orchid, pollination, sequestration, biotransformation, male annihilation technique, sterile-insect technique

INTRODUCTION

The fruit fly tribe Dacini is one of the most species-rich clades within the dipteran family Tephritidae (Diptera) comprising more than 700 species, which includes many fruit pest species of economic importance, particularly in the two genera, *Bactrocera* and *Zeugodacus* [e.g. oriental fruit fly, *B. dorsalis*; banana fruit fly, *B. musae*; guava fruit fly, *B. correcta*; carambola fruit fly, *B. carambolae*; solanaceous fruit fly, *B. latifrons*; peach fruit fly, *B. zonata*; Queensland fruit fly, *B. tryoni*; Japanese orange fly, *B. tsuneonis*; melon fly, *Z. cucurbitae*; cucumber fly, *Z. cucumis*; pumpkin fruit fly, *Z. tau*] [2,16]. Males of many dacine fruit fly species show strong affinity to either methyl eugenol (ME) or cue-lure (CL) (Fig. 1) [20, 42]. Male annihilation technique (MAT) has been developed by using ME as a powerful trapping agent in the eradication and suppression programmes of one of the most destructive species, the oriental fruit fly, *B. dorsalis*, in various countries [15, 21, 32]. In the case of the eradication programme of melon fly, *Z. cucurbitae*, in Okinawa, Japan, CL was used successfully to suppress the initial field population of males prior to release of sterile flies in the sterile-insect technique (SIT) [43]. These lure chemicals are effective only on the male flies (females usually do not respond to the chemicals), but the lure chemicals are potent enough to reduce the male population to such a low level that mating does not occur [32, 43].



Methyl eugenol (**ME**) Cue-lure (**CL**)

Fig. 1. Two major male attractants of *Bactrocera* and *Zeugodacus* fruit flies

The lure activity of ME and CL to male flies are extremely powerful and highly specific to a number of *Bactrocera* and *Zeugodacus* species [20, 42]. However, there are several important pestiferous dacine species, which do not respond to these lures – such as *Bactrocera atrisetosa*, *B. decipiens*, *B. jarvisi*, *B. latifrons*, *B. minax*, *B. pyrifoliae*, *B. tsuneonis* and *Zeugodacus cucumis* (Table 1). We have been seeking new attractants for those 'non-responding' species, particularly those that are endemic agricultural pests. In order to develop new attractants for those male lure-unknown species, we need to understand the nature of fruit flies' life histories, particularly by observing their natural interactions with plant chemicals that have strong affinity with those pest species. Furthermore, we need to study their sexual communication systems, which may involve pheromone precursor materials arisen from specific phytochemicals selectively accumulated by male flies during foraging as in a strong association between *B. dorsalis* and ME-producing plants.

Table 1. Pestiferous *Bactrocera* and *Zeugodacus* fruit fly species – responsive and non-responsive to specific male lures

Methyl-eugenol (ME)-responsive	Cue-lure (CL)-responsive	Non-responsive to ME and CL
B. carambolae	*B. albistrigata*	*B. jarvisi*
B. correcta	*B. frauenfeldi*	*B. latifrons*
B. dorsalis	*Z. caudata*	*B. minax*
B. musae	*Z. cucurbitae*	*B. pyrifoliae*
B. umbrosa	*Z. tau*	*B. tsuneonis*
B. zonata	*Z. tryoni*	*Z. cucumis*

Herein I give an overview of the nature of specific male attractants in association with sex pheromone system, and the flowers as lure source, particularly focusing on synomonal communication between some fruit fly species and so-called "fruit fly orchids" via specific male attractants. Then, I introduce some cases of exploitation of new attractants from plant sources in relation to the male pheromone gland components.

A large part of this study has been conducted as a collaboration project with Dr. Keng-Hong Tan (Malaysia), particularly on fruit fly species of economic importance.

1. Sex pheromone system and male lures

Males of many pestiferous dacine species are known to possess a glandular sac associated with rectum, which is supposed to store and release sex pheromone to attract conspecific females during courtship [6, 42]. The volatile chemical constituents in the rectal glands have been extensively studied in many *Bactrocera* and *Zeugodacus* species, which include aliphatic amides, alcohols, ketones, esters, lactones, spiroacetals, terpenoids, pyrazines, and some aromatic compounds [7]. Many of these volatiles are supposed to be biosynthesized endogenously by males and stored in the rectal gland during sexual maturation. However, the biological role(s) of the male rectal volatiles has not been well understood.

In the case of *B. dorsalis,* the male rectal glands are almost empty with some minor quantities of *N*-3-methylbutylacetamide and a series of fatty acid esters, when the flies were cultured in the laboratory. However, most of the male flies captured in the field sites were found to contain large amounts of two phenylpropanoid compounds, 2-allyl-4,5-dimethoxyphenol (DMP) and (*E*)-coniferyl alcohol (ECF) in large quantities often over 100μg/male (Fig. 2) [24]. DMP and ECF also accumulated in the rectal glands when laboratory-reared males were fed with pure ME [24].

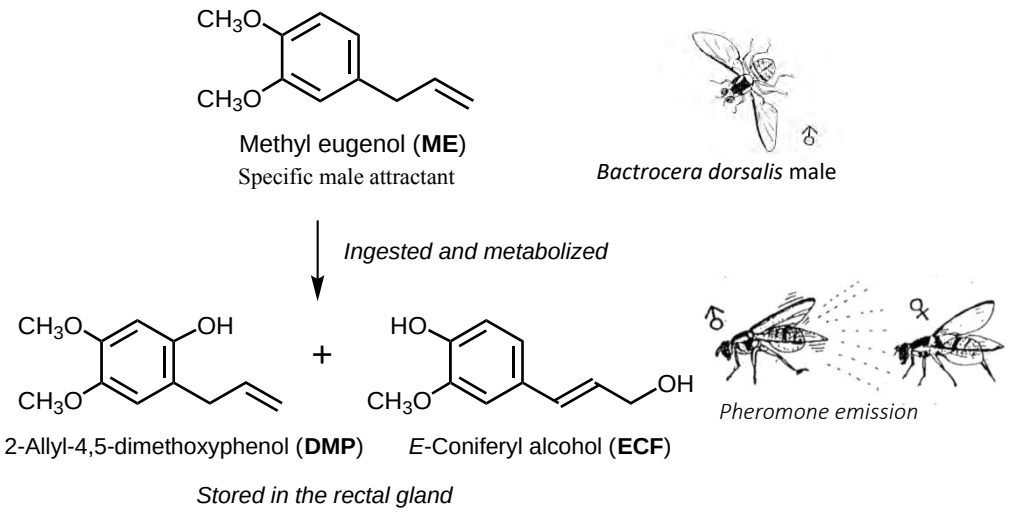


Fig. 2. Methyl eugenol (ME) as a precursor for sex pheromone of the oriental fruit fly, *Bactrocera dorsalis*. Males incorporate ME and convert it to two metabolites

(DMP + ECF) and release them as a "pheromone smoke" to attract and arrest a female during courtship.

These ME-metabolites are released with vigorous wing-fanning action to the air as the "pheromone smoke" from the rectal gland at the sunset when the mating takes place (Fig. 2)[23, cf. 17]. Laboratory studies have shown that *B. dorsalis* males that have fed on pure methyl eugenol have a mating advantage over males denied this compound[9,30,33]. Females are sexually stimulated by the male rectal pheromone components, often exhibiting "ovipositor-extending behavior", possibly a sign of an acceptance of the courting male[33]. However, field trap tests revealed that the rectal metabolites are attractive rather to males but not to females, suggesting that the females are responsive to these compounds only in a very short distance during the courtship period; and males may be triggered to aggregate by the smoke substances (aggregation pheromone) possibly to form a lek prior to courtship[24, 33]. Besides, males that fed on ME were shown to gain a defensive measure (allomone) by sequestering DMP and ECF in their body tissues[23, 34].

A series of related phenylpropanoids, including (*Z*)-coniferyl alcohol (ZCF) and (*Z*)-3, 4-dimethoxycinnamyl alcohol, have been characterized from rectal glands of *B. correcta* and *B. zonata* males compulsively fed on ME[41], suggesting their pheromonal role in their mating sequence. In these instances, the male pheromone systems are suggested to have advanced within the context of sexual selection – the female's preference for the specific components linked to fitness-related defensive compounds[22]. By contrast, males of *B. carambolae* possess significant amounts of endogenous volatiles (e.g. *N*-3-methylbutylacetamide, 6-oxo-1-nonanol, 1,6-nonane- diol) in the rectal glands, and yet 'pharmacophagously' seek the lure source (ME) and sequester it as ECF in substantial quantity; mating advantage by the lure-fed males has been demonstrated[45].

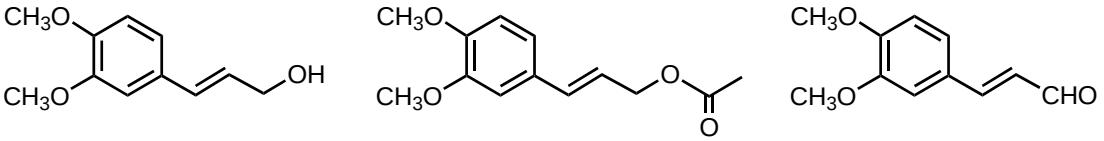
In the case of *Z. cucurbitae*, males fed on CL store its deacetyl compound, raspberry ketone (RK) in the rectal glands together with endogenous volatiles (e.g. *N*-3-methylbutylacetamide, 1,3-nonanediol, ethyl 4-hydroxybenzoate)[25]. *Z. cucurbitae* males fed on either CL or RK were shown to have mating advantage at an early stage after feeding[31].

In the sexual communication system of the olive fruit fly, *B. oleae*, females attract males and the female sex pheromone, 1,7-dioxaspiro[5.5]undecane (olean), has been demonstrated to be useful for bating traps [8]. Although the use of synthetic sex pheromones and other intraspecific chemical communicants is established in lepidopteran species, the use of dipteran pheromones for control or monitoring is not yet common, despite its obvious potential as above. Much detailed work under close scrutiny is required with the dacine fruit fly species to understand the nature of those pheromonal communication systems particularly involving male-specific lure substances.

2. Flowers as lure sources

Males of the lure-responsive fruit fly species frequently visit plants that contain specific chemicals to acquire pheromone materials. Historically, a century ago, ME was found as the powerful male attractant for *Dacus* (*Bactrocera*) species from citronella grass, *Cymbopogon nardus* (Poaceae) [10]. Subsequently, ME was also identified from flowers of golden shower, *Cassia fistula* (Fabaceae), which attracted *B. dorsalis* in Hawaii [13]. The occurrence of ME has been reported for more than 450 species from 80 families spanning many plant orders [38]. Since dacine fruit fly males show strong affinity to some specific floral components, flowers seem to be a good source for the males to acquire these essential chemical components during adult stage.

New attractants for *B. dorsalis* males were isolated from a Hawaiian lei flower, *Fagraea berteriana* (Gentianaceae), and characterized as a mixture of (*E*)-3,4-dimethoxycinnamyl alcohol, its acetate, and (*E*)-3,4-dimethoxycinnamaldehyde [27] (Fig. 3). A similar set of compounds was also found from flowers of *Spathiphyllum cannaefolium* (Araceae) [1]. Since ME is suspected as a human carcinogen [12], these non-allylic compounds might potentially serve as its substitutes in place of ME in the pest management programmes.



3,4-Dimethoxycinnamyl alcohol 3,4-Dimethoxycinnamyl acetate 3,4-Dimethoxycinnamaldehyde

Fig. 3. Attractants for *Bactrocera dorsalis* males from flowers of *Fagraea berteriana*

Above naturally occurring lure chemicals are classified as phenylpropanoids (C6-C3) closely related to ME. By contrast, cue-lure (CL) is a phenylbutanoid (C6-C4) that has never been found from the natural sources (developed as a synthetic attractant; but its deacetyl derivative, raspberry ketone (RK) was found in nature – from the flower of *Dendrobium superbum* (Orchidaceae) as a specific attractant for *Z. cucurbitae* [26].

Our systematic survey on flowers of so-called "fruit fly orchids" in the orchid genus *Bulbophyllum* (Orchidaceae) revealed a series of phenylpropanoids and phenylbutanoids that attract specific fruit fly species. Flowers of *Bu. cheiri* attract males of several *Bactrocera* fruit fly species by floral ME in the rain forest of Southeast Asia [28, 39] (Fig. 4, left). The flower has a specialized hinged see-saw lip structure which temporarily traps the fruit fly between its lip and column, and transfers pollinia to the back of his thorax. The fly is rewarded by the floral attractant to be used as a precursor for sex pheromone and defensive allomone as described in Section 1 (see Fig. 2). In this orchid-fruit fly association, both organisms gain direct reproductive benefits – the orchid flower gets pollinated without having to offer nectar, while the fruit fly boosts its pheromone with the attractant chemical ME. Thus, the floral volatile is defined as "synomone", an interspecific semiochemical that is adaptively advantageous to both the emitting and the receiving organisms. Flowers of another related orchid species, *Bulbo. vinaceum*, produce a complex mixture of phenylpropanoids including DMP and ECF, in addition to ME and attract ME-sensitive fruit flies [40]. The orchid flower seems to endow the male flies not only with precursor attractant ME but also intact pheromone (DMP and ECF), although the complete pollinator spectrum and actual role of such a multi-component system of attractant volatiles is unclear.

Another fruit fly orchid, *Bulbo. apertum* (synonym: *ecornutum*), RK in its fragrance to attract several RK-sensitive *Bactrocera*/*Zeugodacus* species such as *B. albistrigata* and *Z. cucurbitae* (Fig. 4, right) [36]. The pollination takes place when a fly is compulsively feeding on the seesaw lip and momentarily trapped between the lip and column. The attractant chemical can act either as floral synomone (pollinarium-transported, as in *B. albistrigata*) or kairomone (accidental removal of pollinarium leading to total pollen wastage, as in *Z. cucurbitae*) depending on the size of the visiting fruit fly species [36].

Flowers of *Bulbo. patens* attract male of a variety of *Bactrocera* fruit fly species, which show affinity to either ME and RK. The attractant component was identified as zingerone [4-(4-hydroxy-3-methoxyphenyl)-2-butanone] (ZN), a pungent essence of ginger (Fig. 4, middle) [35]. ZN conforms to a chemical structure resembling both ME and RK, and thus shows a potency to attract both ME-sensitive and RK-sensitive fruit fly species (e.g. *B. carambolae*, *B. dorsalis*, *B. umbrosa*, *Z. caudata* and *Z. cucurbitae*), thereby securing effective pollinators in the complex and dense vegetation in the rain forests. It was demonstrated that *B. cucurbitae* males that have fed on ZN sequester the intact compound in the rectal pheromone glands and become more successful in mating than unfed males [14]. Interestingly, males of an Australian fruit fly, *B. jarvisi* (a pest of mango fruit) whose specific attractant was initially unknown, was found to be selectively attracted to ZN in the flowers of *Bulbo. baileyi* that the male flies frequently visit [4, 37]. ZN was also attractive to other species such as *B. aglaiae* and a new species *B. speewahensis* and the rarely trapped *Dacus secamoneae* in the field of northern Australia [4]. This exemplifies a possible use of floral synomones as new monitoring agents for several specific pest species in the orchards.

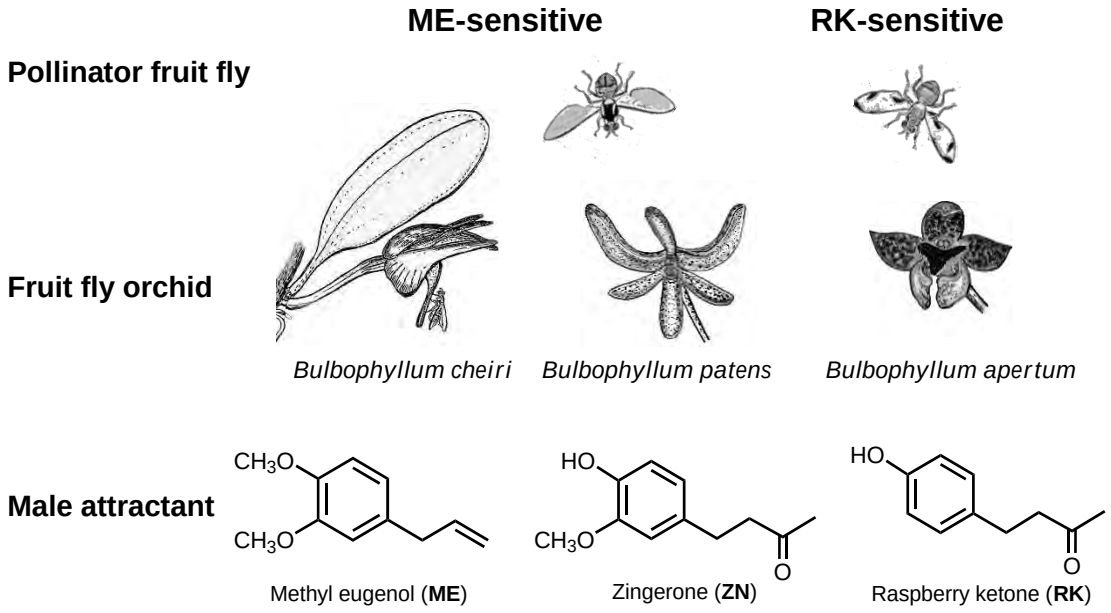


Fig. 4. Floral synomone of fruit fly orchids attracting pollinator fruit fly males.

Note: *Bulbo. vinaceum* emits methyl eugenol (ME); *Bulbo. apertum* produces raspberry ketone (RK); *Bulbo. patens* emits zingerone (ZN) which has a hybrid structure between ME and RK and attracts both ME-sensitive (e.g. oriental fruit fly, *B. dorsalis*) and RK-sensitive species (e.g. melon fly, *Z. cucurbitae*).

3. New attractants derived from male rectal components

3-1. *Bactrocera latifrons*

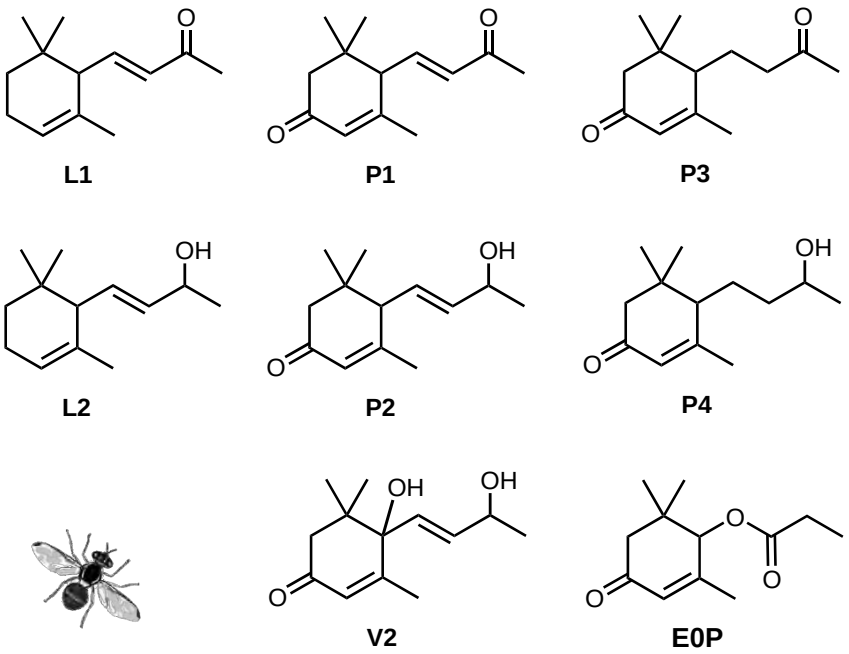
Among the pest fruit fly species with unknown male attractants, α-ionone (L1) and α-ionol (L2) (latilure) were found as specific attractants for the solanaceous fruit flies, *B. latifrons*, during the chemical screening using olfactometry (Fig. 5)[5]. Although the attractiveness of these norsesquiterpenes was weak, the lure activity was significantly enhanced by cade oil[18, 19]. However, the potency of these attractant mixtures appeared not as high as that of ME for *B. dorsalis* or CL for *B. cucurbitae*. Some efforts were put into a survey for potential chemical lures for *B. latifrons* particularly in orchids, but observations showed none of the flies settled on any plant sources.

During the search for solanaceous host fruit volatiles to attract *B. latifrons* females, a variety of eggplants commonly sold in a grocery store in Hawaii was found to strongly attract males of *B. latifrons* rather than females[29]. The males voraciously feed on the eggplant tissues and its extracts, similar to the phenomenon shown by *B. dorsalis* males compulsively licking on ME-containing plants. One of the active principles was identified as 3-hydroxy-α-ionone[29].

Isophorone (3,5,5-trimethylcyclohex-2-enone) and its analogs were also found as moderate attractant for *B. latifrons* males, which significantly enhanced activity of L1 and L2[11]. Based on that knowledge, a series of 3-oxygenated α-ionone analogs (P1, P2, P3 and P4) have been prepared, and their individual activities were evaluated (Fig. 5). Synthetic 3-oxo-α-ionone (P1) showed distinct attractant activity in the laboratory tests; 7,8-dihydro-3-oxo-α-ionone (P3) was shown to be effective in the field tests using Steiner traps[3, 11]. It should be noted that all 3-oxygenated analogs showed potent phagostimulant activity as well as long-range attraction, which appeared typical properties of male lures as in the case of *B. dorsalis* to ME and *Z. cucurbitae* to CL and RK.

P3 has been selected as a possible candidate for use as a monitoring agent for *B. latifrons* in Okinawa (unpubl. data). Further survey also revealed vomifoliol (V2) – isolated from roots of lamb's ear, *Stachys byzantina* (Labiatae), was found to be an extremely potent phagostimulants for the male flies, although long distance attractiveness was very weak, due to its low volatility (unpubl. data) (Fig. 5). Along with the above information, several analogs with an oxygen function on the C-4

position of isophorone have been synthesized to improve the attractiveness. As a result of outdoor cage tests, 4-propionyloxyisophorone (EOP) has been developed as a powerful lure against *B. latifrons*, particularly more effective when used in combination with P3 (unpubl. data) (Fig. 5). EOP is a propionate of 4-hydroxy- isophorone (EO), and the ester evaporates much faster than P3 at ambient temperature, even though they possess almost similar molecular sizes.



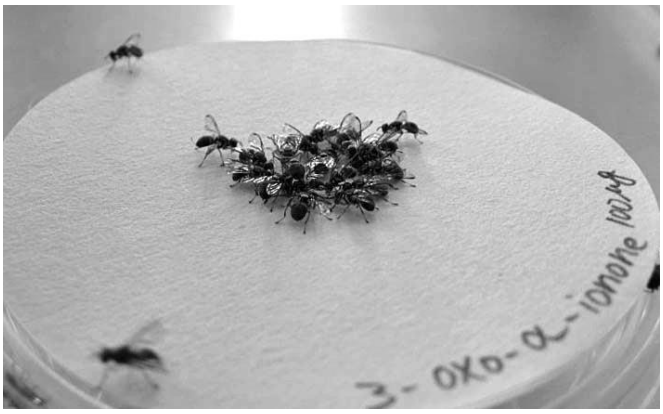
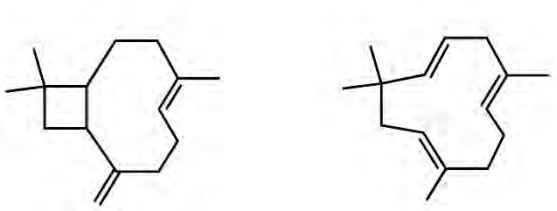


Fig. 5. Structures of attractants/phagostimulants of α-ionone/ionol analogs for the solanaceous fruit fly, *Bactrocera latifron*s. Photo shows a congregation of the male flies attracted and voraciously feeding on 3-oxo-α-ionone (P1).

3-2. *Bactrocera correcta*

B. correcta males are attracted to ME and sequester *Z*-3,4-dimethoxycinnamyl alcohol and ZCF in the rectal glands as mentioned above (Section 1). Besides these two ME-metabolites, wild males were found store extremely large quantities of sesquiterpenic hydrocarbons, such as β-caryophyllene (CP) and α-humulene (HM) in the rectal glands in a survey of local *B. correcta* flies in Thailand. Total amounts of sesquiterpenes often exceeded 250 μg/gland (Fig. 6)[44]. CP is a common plant essential oil component in such as clove (*Syzygium aromaticum*), rosemary (*Rosmarinus officinalis*), lavender (*Lavandula angustifolia*), hops (*Humulus lupulus*), and *Cannabis sativa*.

Field tests were conducted using one of the rectal components – CP, in fruit orchards in Thailand. CP was shown to act as a highly potent male attractant for *B. correcta*, in that its attractiveness appears to be significantly higher than that of ME, whereas the co-inhabiting *B. dorsalis* was never captured by CP-traps[46]. *B. correcta* males respond to CP earlier than their attraction to ME nearing or at sexual maturity[46]. This is highly desirable in a mass-trapping or male annihilation program to reduce mating of feral females. In a monitoring program of both *B. correcta* and *B. dorsalis*, CP can be employed as a useful measure to differentiate the trap catches of each species, or possibly as a highly selective mass trapping agent for *B. correcta*.



β-Caryophyllene (**CP**) α-Humulene (**HM**)

Fig. 6. Sesquiterpene lures specific for *Bactrocera correcta* – identified from the fly male rectal glands

Sequestration of these sesquiterpenes in the pheromonal gland in *B. correcta* males poses a great contrast to that in *B. dorsalis* males, suggesting a potential role of these unique rectal ingredients in the interspecific interactions between these sympatric

species. It has been demonstrated that CP-feeding increased the male mating success in comparison with lure-deprived males, and CP-fed males were equally competitive as ME-fed males when competing for conspecific females in semi-field trials [46].

CONCLUSION

The strong attractiveness to plant allelochemicals is a unique characteristic in *Bactrocera/Zeugodacus* males. We demonstrated the nature of sex pheromone system in conjunction with specific male lure, and interpreted why those male fruit flies are strongly attracted and eagerly feed on the lure chemicals. We successfully found a series of new male specific lures by chemical analyses of flower fragrances in fruit fly attracting plants particularly in 'fruit fly orchids' in tight mutual association with the dacine fruit flies, rectal pheromone gland volatiles from wild males of target fruit fly species, and structural modifications of such molecules through organic syntheses. The strong affinity of fruit flies to these lure chemicals seems to be linked to the flies' pheromone and defensive systems specifically developed within the highly diverged species radiating from central Southeast Asia. We need to pursue further to understand the natural life history of these fruit flies in close association with plants in their natural and agricultural ecosystems.

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